Determination of Pesticides in Drinking Water by Solid Phase Extraction and Liquid Chromatography / Mass Spectrometry

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A method was developed based on a United States Geological Survey (USGS) method (1) to determine a number of pesticides in drinking water. A graphitized carbon solid phase extraction (SPE) cartridge is used to isolate pesticides from drinking water. Residual chlorine is removed prior to extraction. The water is acidified to enhance the retention of acidic compounds and salt is added to improve overall recoveries. Pesticides are eluted with methanol followed by an acidic mixture of methylene chloride / methanol followed by acetone. A high performance liquid chromatograph (HPLC) coupled with a triple quadropole mass spectrometer (MS) with multiple reaction monitoring (MRM) is used to identify, quantitate and confirm pesticides isolated by the procedure.

Reagents

- Methylene Chloride, J.T. Baker "Ultra Resi-analyzed"
- Acetone, J.T. Baker "Ultra Resi-analyzed"
- Methanol, (MeOH) , J.T. Baker "Baker-analyzed", HPLC grade
- Sodium Chloride, (NaCl), Fisher Chemicals, Certified ACS
- Glacial Acetic Acid, Fisher Chemicals, Certified ACS plus
- Hydroxylamine Hydrochloride, (NH_2OH), Fisher Chemicals Certified ACS
- Orthotolidine dihydrochloride (o-Tolidine)
- Hydrochloric Acid, (HCL)
- Trifluoroacetic acid (TFA)
- Nitrogen gas, 99.99% pure
- Distilled De-ionized water (DDI)

Solutions

- 1% NH_2OH - Add 10g Hydroxylamine Hydrochloride to a 1 L volumetric. Dissolve and bring to volume with DDI.

- o-Tolidine reagent. Dissolve 0.135g o-Tolidine in 50ml DDI. Add with stirring, a mixture of 35ml DDI and 15ml HCL. Store in an amber bottle for no longer than 6 months.

- 80/20 Methylene Chloride / Methanol. Add 200ml Methanol to 800ml Methylene Chloride. Add 2ml TFA and mix well.

Apparatus

- Supelclean ENVI-carb SPE cartridge, 6 ml x 500 mg. Supelco, part # 57094.
- Supelco Visidry attached to nitrogen source.
- Zymark TurboVap attached to nitrogen source.
- Zymark concentrator tube, 200ml .
- Kuderna Danish (KD) receiving tubes, 10ml. Tubes are graduated to 1ml in 0.1ml increments. 0.5ml mark should be calibrated and permanently etched.
- Zymark Autotrace workstation.
- Vortex genie.

Precautions

Fire all glassware used in the procedure at 450 ^{o}C for 2 hours.

Analytical Instrument

LC/MS/MS-Water's 2690 HPLC with a Micromass Ultima Mass Spectrometer.
Water's XTerra MS C-18 column, 2.1 x 150mm

	Solvent Gradient	
Time	% A (0.15% Acetic Acid)	%B (MeOH)
0.00	86.0	14.0
0.50	86.0	14.0
30.00	45.0	55.0
45.00	10.0	90.0
50.00	10.0	90.0
50.50	86.0	14.0
55.00	86.0	14.0

Sample Pickup and Transport

Samples are obtained by personnel at selected municipal water plants. Each sample is collected in a 1000 ml amber glass bottle containing 1 ml of 1% NH_2OH and fitted with a Teflon lined screw cap. The sample is refrigerated and transported to the lab within 24 hours of pickup.

Sample Reception

1. Samples are to be kept refrigerated until the time of analysis.

2. Remove 5ml water and test for residual chlorine with 2 drops o-Tolidine reagent.

3. A yellow color indicates the presence of chlorine.

4. Add 1% NH_2OH in 0.5ml increments until test for residual chlorine is negative.

5. Weigh the sample plus sample bottle.

Analytical Procedure

1. Samples are to be analyzed within three days of sample receipt or within four days of sample pickup.

2. Re-weigh sample bottle after sample has been extracted to determine sample weight by difference.

3. Weigh a 6 ml x 500 mg ENVI-carb SPE cartridge and record weight on cartridge.

4. Condition SPE cartridge with 2 x 5ml 80/20 Methylene Chloride / Methanol followed by 2 x 5ml DDI. DO NOT ALLOW THE CARTRIDGES TO GO DRY.

5. Attach the conditioned cartridges to the Zymark Autotrace.

6. Add 10g NaCL to 1000 ml sample and mix until dissolved.

7. Add 1.5ml Acetic Acid to 1000 ml sample and mix well.

8. Add process control and /or spiking solutions at this step and mix well.

9. Pump water samples through the conditioned cartridges at a flow rate of 25ml/min.

10. Remove cartridge from Zymark and place on Supelco Visidry. Dry cartridge with nitrogen until original weight is attained.

11. Elute cartridge by gravity into a 200ml Zymark tube with 2ml MeOH, followed by 2 x 5ml 80/20 Methylene Chloride/Methanol, followed by 5ml Acetone.

12. Apply pressure to ensure that all Acetone is removed from cartridge.

13. Concentrate the eluants to approx. 0.1ml using the TurboVap.

14. Transfer the concentrated eluant to a calibrated KD receiving tube.

15. Rinse the Zymark tube with a small amount of MeOH and transfer to the KD tube.

16. Concentrate the solution to 0.1ml.

17. Add water to bring the volume to 0.5ml and mix using a vortex mixer.

18. Transfer to auto sampler vial for LC/MS/MS analysis

Zymark Autotrace Cleanup

After each sample has been pumped thru the Zymark Autotrace, clean the components in the following manner

1. Pump 30 ml of a mixture of 10% Toluene, 20% Methylene Chloride and 70% Isopropanol thru the various lines and fittings of the Autotrace at a flow rate of 10ml / min.

2. Pump 30 ml MeOH thru the Autotrace at a flow of 10 ml / min.

3. Pump DDI thru the Autotrace at a flow of 10 ml / min.

Process Control

Propoxur is to be used as the process control for this extraction. 100ul of a 2.5 ppm solution of propoxur when added to each 1000ml sample will result in a concentration of 0.25 ppb (250 ppt) or approximately five times the limit of quantitation (LOQ).

Spike Samples

Place 1000ml of a water sample, known to be free of the pesticides under study, in a clean container. Add 1ml 1% NH_2OH and mix. Add 10g NaCl and 1.5ml Acetic acid and mix. Add 100ul, 200ul, 500ul or 1ml of a prepared solution of the pesticides under study so that the resulting levels of pesticides correspond to 1, 2, 5, or 10 x LOQ.

Standards

Standard are prepared in matrix by the following procedure to encompass the linear range from the 1 x LOQ to 10 x LOQ.

To make 8 LC standards in matrix (4 LC1, 4LC2):
Run six 1 liter aliquots of tap water through the LC method (Determination of Pesticides in Drinking Water by Solid Phase Extraction and Liquid Chromatography /Mass Spectrometry) steps 1-14. Rinse 5 of the Zymark tubes into the 6^{th} Zymark tube using MeOH. Concentrate this to 600 ul. Put a 50 ul aliquot into a microvial insert in an autosampler vial. Blow the 50 ul to dryness, then add 250 ul of each standard, cap, and vortex.

Standard curves are prepared for each group of samples run.

Quantitation

The level of pesticide in each 0.5ml assay solution is determined by use of a standard curve or by direct comparison of standards that fall within + /- 30% of the response of the sample.

$$\text{Sample (ppt)} = \frac{\text{Assay (ppm)} \times 0.5\text{ml} \times 10^6}{\text{WT sample(g)}}$$

References

1. Methods of Analysis by the U S Geological Survey National Water Quality Laboratory – Determination of Pesticides in Water by Carbopak–B Solid Phase Extraction and High-Performance Liquid Chromatography. Open-File Report 96-216.